

## GUEST COMMENTARY

### Facultative Methanotrophs Revisited

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Methane is one of the most important greenhouse gases, and its concentration in the atmosphere is increasing by approximately 1% per year (16). Methane-oxidizing bacteria, or methanotrophs, are a key group of bacteria involved in the global methane cycle and can be found in many environments. For example, they limit the efflux of methane produced in flooded soils and wetlands and consume atmospheric methane directly in aerated upland soils (2, 14). Methanotrophs have generally been considered to be obligate in nature, i.e., growing only on methane as their sole source of carbon and energy. In this issue of the *Journal of Bacteriology*, Dedysh and colleagues provide the first unequivocal proof of a genus of methanotrophic bacteria (*Methylocella*) that are capable of growth on a number of multicarbon substrates, dispelling the notion that methanotrophy is an obligate phenomenon (6). In a century of research since the Dutch microbiologist Söhngen described the first methanotroph, *Bacillus methanicus*, in 1906 (25), significant advances in understanding the ecology and physiology of these organisms have contributed to our understanding of methane cycling in the environment. A brief history of the field will allow the reader to appreciate the nature of this highly controversial topic in the field of bacterial one-carbon metabolism.

Claims for the existence of facultative methanotrophs (i.e., methanotrophs capable of growth on multicarbon as well as one-carbon substrates) have a long and somewhat checkered history dating back almost 30 years, when Patt et al. first isolated (22) and later described (23) *Methylobacterium organophilum*, which was able to grow on methane or glucose. This was followed by other reports of facultative methanotrophs, notably strain R6, *Methylobacterium ethanolicum*, and *Methylomonas* sp. strain 761M (18, 21, 32). In most cases, either the methane-oxidizing capacity was lost or the results were not substantiated in other laboratories. For example, for *M. organophilum* it was hypothesized that methane oxidation genes were plasmid encoded and that the loss of growth on methane was attributable to loss of a plasmid. In the case of *M. ethanolicum*, the culture was shown to be a very tight syntrophic association between a methanotroph and a *Xanthobacter* species (17). The controversy surrounding facultative methanotrophs was recently highlighted by the description of a “methane-oxidizing” methylotroph, *Methylobacterium populi*, and the rebuttal that followed (5, 28). Thankfully, doubts about

the existence of facultative methanotrophs seem finally to have been dispelled by the meticulous experiments performed by Dedysh et al. (6). Quantitative real-time PCR targeting the *mmoX* gene, which encodes the hydroxylase component of the soluble methane monooxygenase (sMMO), the key enzyme for methane oxidation in *Methylocella silvestris* BL2, showed a parallel increase of copies of *mmoX* and microscopic cell counts in both methane- and acetate-grown cultures. In addition to fluorescence in situ hybridization using strain- and genus-specific oligonucleotide probes, most-probable-number dilution experiments ruled out the possibility of a contaminant present at low numbers which could catabolize the acetate and provide a carbon source for the dominant *Methylocella* population.

The cause or causes of obligate methanotrophy remain elusive despite decades of research on this subject, although several hypotheses have been investigated (31). Experiments performed on whole-cell suspensions and cell extracts have shown that the failure of obligate methanotrophs to grow on multicarbon substrates such as acetate, as well as amino acids and sugars, was not necessarily due to their inability to transport the substrates into the cell (11, 12, 24). The observation of specific metabolic lesions in methanotrophic bacteria, such as the absence of alpha-ketoglutarate dehydrogenase activity in type I methanotrophs, is the most likely explanation, although the question remains as to how or why these lesions arose. To complicate things further, the recently released genome sequence of *Methylococcus capsulatus* (Bath) revealed that the genes encoding alpha-ketoglutarate dehydrogenase were indeed present and apparently complete, although there is as yet no evidence for expression of an active enzyme (29, 31). Type II methanotrophs such as *Methylocella* have also been shown to lack activity of the glyoxylate cycle enzymes isocitrate lyase and malate synthase (10). Therefore, we can only speculate about whether *Methylocella* species use an alternative pathway for growth on acetate or whether appropriate enzyme assay conditions have not yet been found. Another interesting observation is the apparent reduced growth yields of *Methylocella* grown on methane as the sole substrate compared to other serine pathway methanotrophs. This may indicate a reduced efficiency of energy generation from methane, but further investigation will be necessary to determine if this is the price a facultative methanotroph pays for being more metabolically versatile.

Our outlook on methane cycling in the environment may also be outdated: our previous understanding of methanotroph physiology did not take into account the effect of multicarbon substrates on methanotrophic activity. This is highlighted by

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the authors' observations on the addition of acetate to a methane-grown culture of *Methylocella*: although the initial effect of acetate addition was to inhibit methane oxidation, the longer-term consequence was a stimulatory effect on methane oxidation (6). Previously, the positive effect of acetate addition to a tundra soil on methane oxidation was attributed to increased activity of methanogens, resulting in an indirect stimulation of the methanotrophic community (30). The discovery of facultative *Methylocella* species suggests that these findings need to be reappraised. Indeed, acetate is a major intermediate in carbon turnover in soil and is a major end product of anaerobic metabolism in wetlands (9, 15). *Methylocella* inhabits acidic wetlands and forest soils, which account for a large proportion of methane emissions globally (8, 13, 19), and thus *Methylocella* and potentially other, as-yet-unknown facultative methanotrophs may be major players in methane cycling in these environments and thus globally. There have been no reports on the isolation of methanotrophs capable of oxidizing methane at atmospheric concentrations, but it is tempting to speculate that since Dedysh et al. have shown that the threshold of methane oxidation was lowered following acetate addition in *Methylocella* (6), this type of mechanism might enable other, as-yet-undescribed facultative methanotrophs to oxidize methane at atmospheric concentrations.

These recently confirmed metabolic traits may also offer interesting new biotechnological potential for facultative methanotrophs such as *Methylocella*. *Methylocella* apparently possesses only the sMMO and not the particulate MMO (pMMO), which has been found in all other methanotrophs thus far examined (4, 7, 10, 20). The sMMO enzyme, which is found in some but not all methanotrophs that also possess a pMMO, has attracted considerable attention due to its catalytic versatility and the ability to oxidize a wide range of substrates, including alkanes, alkenes, alicyclics, aromatics, ethers, and heterocyclics (1) and several important environmental contaminants such as the halogenated hydrocarbons trichloroethylene and chloroform. These compounds are not carbon and energy sources for methanotrophs but are fortuitously cooxidized by sMMO (3, 27). In all other methanotrophs containing sMMO, copper-mediated regulation results in expression of sMMO only under low copper-to-biomass ratio growth conditions, while pMMO is expressed under high copper-to-biomass ratio conditions (26). The pMMO, in comparison to the sMMO, however, cooxidizes only a relatively narrow range of substrates such as short-chain alkanes and alkenes, in addition to oxidizing methane. Thus, *Methylocella* is potentially attractive from a biotechnological viewpoint in that expression of sMMO in this methanotroph might not be repressed by copper ions in the environment. One could easily envisage the benefits of stimulating indigenous or augmented *Methylocella* populations with acetate for bioremediation of contaminated sites. It is noteworthy, however, that the lack of pMMO in *Methylocella* remains a contentious issue, and the evidence presented thus far by Dedysh et al. (7) seems to be insufficient to rule out completely the presence of pMMO or an alternative methane-oxidizing system to sMMO in *Methylocella*.

In summary, Dedysh et al. have made an important contribution in the field of one-carbon metabolism by describing the first fully authenticated facultative methanotroph (6). This unique capacity to grow on multicarbon substrates has major

implications for our outlook on methane cycling in the environment and the notion that methanotrophy is an exclusively obligate phenomenon. Future work addressing the molecular physiology of these organisms, particularly the regulation of the methane-oxidizing machinery during growth on multicarbon substrates, is essential to advance our understanding of methane cycling in the environment and to improve the possibilities of exploiting the unique characteristics of *Methylocella* and other facultative methanotrophs. The availability of the genome sequence of *M. silvestris* BL2 would further enhance such molecular physiological studies.

We gratefully acknowledge D. P. Kelly for his comments on the manuscript.

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